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AGGLUTINATION TEST AS A MEANS OF STUDYING THE PRESENCE OF BACTERIUM ABORTUS IN MILK

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INTRODUCTION

In the investigation of the effect on milk of the diseases of the cow, with special reference to infectious abortion, it was found desirable to examine a large number of samples to determine whether or not *Bacterium abortus* Bang was being passed with the milk. The cultural and animal-inoculation methods were the only ones found available for this work.

The cultural method devised by Nowak [1] takes advantage of the fact that newly isolated cultures require an atmosphere partially depleted of oxygen. This atmospheric condition is obtained by growing the agar streaks from suspected material in a closed jar with *Bacillus subtilis*, having 1 sq. cm. of culture surface to each 15 c. c. of jar capacity. While the author has isolated *Bact. abortus* from milk sediment by this method, it is too tedious a process to apply to any number of samples. Plates are likely to be overgrown with colonies of fast-growing organisms, and the method has the further disadvantage of requiring several weeks to isolate and identify the cultures.

Evans [2] succeeded in isolating *Bact. abortus* from milk by plating on ordinary lactose agar to which 10 per cent of sterile blood serum was added just before plating. After incubating for four days, the colonies which developed were transferred to nutrient broth containing 1 per cent glycerin and to tubes of whole milk containing litmus.

The other method of study, the inoculation of guinea pigs with the milk, while more reliable, is far from satisfactory, owing to the fact that it takes 8 to 10 weeks for the lesions to develop, and it is probable that the organism must be present in large numbers to cause the characteristic lesions with the 5 c. c. of milk used for inoculation.

[1] Nowak, Jules. Le bacille de Bang et sa biologie. *In* Ann. Inst. Pasteur, t. 22, no. 6, p. 541–556, pl. 5–7. 1908.

[2] Evans, Alice C. Bacillus abortus in market milk. *In* Jour. Wash. Acad. Sci., v. 5, no. 4, p. 122–125. 1915.

In studying the presence of *Bact. abortus* in milk it was found necessary to develop new technic in order to study a large number of samples. Knowing that this organism is sometimes present in considerable numbers in milk as it comes from the cow's udder, it was thought that this might indicate an infection of the udder and a consequent local production of antibodies. With this in mind, agglutination and complement-fixation tests were made, using milk and milk serum, instead of the usual method of using blood serum. *Bact. abortus* was used as antigen. The object of this paper is to report upon this method.

TECHNIC EMPLOYED

COMPLEMENT-FIXATION TEST.—The complement-fixation test as used by Surface [1] and others, was employed in this work. Rennet milk serum was used in the following quantities: 0.1, 0.04, 0.02, and 0.005 c. c. Milk was considered positive only when the tube containing 0.04 c. c. of serum was positive. Preliminary tests run upon samples of milk show that the agglutination and complement-fixation tests correspond closely. For this reason only the results of agglutination tests will be given in this paper.

AGGLUTINATION TEST.—Antigen was prepared for the agglutination test by growing a culture of *Bact. abortus* upon ordinary agar for 48 hours. The growth was then washed off with a solution containing 0.9 per cent sodium chlorid and 0.5 per cent phenol. The suspension was then filtered through a coarse filter paper and standardized so that the turbidity compared with tube 1.5 of McFarland's nephelometer.[2] Four c.c. of this bacterial suspension are placed in each of the small test tubes used and the following quantities of milk added: 0.1, 0.05, 0.025, 0.01, and 0.005 c. c. In this way approximate dilutions of 1 to 50, 1 to 100, 1 to 200, 1 to 500, and 1 to 1,000 were obtained. It was found that turbidity due to the whole milk added did not interfere with the reading of the reaction. When a dilution lower than 1 to 50 was made, rennet milk serum was used.

For the experiment given in Table I, a cow was selected whose milk had given a negative agglutination reaction since first tested, Octobe 10, 1914, using *Bact. abortus* as antigen. Thirty-five c. c. of a 48-hou broth culture of *Bact. abortus* was introduced into the right rear quarte after it had been milked dry. As shown in the table, the agglutinin had appeared in the right rear quarter the following day and soon sprea to the other quarters. This spreading was probably brought about b the organism being carried from quarter to quarter upon the hands duri milking. After the cow freshened the reaction was seen to gradua die out.

[1] Surface, F. M. The diagnosis of infectious abortion in cattle. Ky. Agr. Exp. Sta. Bul. 166, p. 301- 5 fig. 1912.

[2] McFarland, Joseph. The nephelometer. . . In Jour. Amer. Med. Assoc., v. 49, no. 14, p. 1176 2 fig. 1907.

TABLE I.—*Test showing the appearance in milk of agglutinins for Bacterium abortus after the introduction into the cow's udder of a pure culture of Bact. abortus Bang* [a]

[Agglutination reaction at middle of milking, when various quantities of milk were added to test tubes containing bacterial suspension]

Date.	Right rear quarter. 0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	Right front quarter. 0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	Left rear quarter. 0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	Left front quarter. 0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.
1914. Oct. 10	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
1915. Feb. 8	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
15	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
24	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
25	(b)	(b)	(b)	(b)	(b)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
26	+	P	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
27	P	P	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Mar. 1	+	+	+	−	−	+	P	−	−	−	+	P	−	−	−	+	P	−	−	−
2	+	+	−	−	−	+	P	P	−	−	+	+	+	P	−	+	P	P	−	−
5	+	+	−	−	−	+	+	P	−	−	+	+	+	P	−	+	+	P	−	−
8	+	+	+	−	−	+	+	+	P	−	+	+	+	−	−	+	+	+	−	−
9	(c)	(c)	(c)	(c)	(c)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
10	+	P	−	−	−	+	+	−	−	−	+	P	−	−	−	+	P	−	−	−
11	+	−	−	−	−	+	−	−	−	−	+	−	−	−	−	+	−	−	−	−
Apr. 17	+	+	P	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
May 26	+	P	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
June 4	P	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
July 28	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Aug. 21	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Sept. 13	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

[a] The +, −, and P signs used in all the tables refer to agglutination reaction in the corresponding tube. For instance, +++P− indicates that agglutination took place in the tubes containing 0.1, 0.05, and 0.025 c. c. of milk, partial agglutination took place in the tube containing 0.01 c. c. of milk, and there was no agglutination in the tube containing 0.005 c. c. of milk.

In all cases, unless otherwise stated, the milk was taken a little before what was estimated to be the middle of the milking.

[b] 35 c. c. of a 48-hour broth culture of *Bact. abortus* introduced into right rear quarter.

[c] Cow calved. Bull calf died on Mar. 13, 1915, owing to undigested curd. Reaction of blood of calf; −agglutination; +complement-fixation test.

Table II gives the history of milk from a cow with a record of frequent abortions. As shown in the table, the isolation of *Bact. abortus* from the milk and the results of guinea-pig inoculation prove the presence of this bacterium, as indicated by agglutination reactions.

TABLE II.—*History of milk from a cow with a record of frequent abortions* [a]

[Agglutination reaction at middle of milking, when various quantities of milk were added to test tubes containing bacterial suspension]

Date.	Right rear quarter. 0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	Right front quarter. 0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	Left rear quarter. 0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	Left front quarter. 0.01 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.
1914. Jan. 10 [b]	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Apr. 30	+	+	+	−	−	+	+	P	−	−	+	+	+	P	−	+	+	+	+	−
May 5	+	+	+	−	−	+	+	P	−	−	+	+	−	−	−	+	+	+	−	−
June 20	+	+	+	P	−	+	+	+	−	−	+	+	+	P	−	+	+	+	P	−
July 11	+	−	−	−	−	+	P	−	−	−	+	+	+	P	−	+	P	−	−	−
12 [c]	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Aug. 10	+	+	−	−	−	+	+	+	P	−	+	+	+	P	−	+	+	+	P	−
28	+	+	−	−	−	+	−	−	−	−	+	+	−	−	−	+	+	−	−	−
Oct. 10	+	+	−	−	−	+	+	−	−	−	+	+	+	−	−	+	+	+	−	−
31	+	+	P	−	−	+	+	+	−	−	+	+	+	P	−	+	+	+	−	−
Nov. 19 [d]	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

[a] Known abortions: Dec., 1909; Jan., 1914. Jan., 1911, last living normal calf. Other records of abortions lost.

[b] Isolated a pure culture of *Bact. abortus* direct from milk.

[c] Guinea pigs inoculated intra-abdominally with milk from each quarter had typical *Bact. abortus* lesions when autopsies were performed eight weeks later.

[d] Died; impaction of stomach. No lesions or abnormal conditions found in udder.

In Table III the record of milk from another cow is given. Here again we have positive agglutination coupled with abortions and milk shown to contain *Bact. abortus* by guinea-pig inoculation.

TABLE III.—*History of milk from a cow with a record of frequent abortions*

[Agglutination reaction at middle of milking, when various quantities of milk were added to test tubes containing bacterial suspension]

Date.	Right rear quarter.					Right front quarter.					Left rear quarter.					Left front quarter.				
	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.
1914.																				
July 20	+	P	–	–	–	+	+	P	–	–	+	+	–	–	–	+	P	–	–	–
Aug. 4[a]																				
10	+	+	–	–	–	+	+	–	–	–	+	+	+	–	–	+	+	–	–	–
24	+	+	+	–	–	+	+	+	–	–	+	+	–	–	–	+	+	–	–	–
Oct. 1	+	+	+	–	–	+	+	P	–	–	+	+	+	–	–	+	+	+	–	–
Nov. 27[b]																				
27	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dec. 2	+	+	+	–	–	+	+	+	–	–	+	+	+	–	–	+	+	+	–	–
1915.																				
Jan. 15	+	+	P	–	–	+	P	–	–	–	+	P	–	–	–	+	–	–	–	–
Mar. 25	P	P	–	–	–	P	P	–	–	–	P	P	–	–	–	P	P	–	–	–
June 8	P	P	–	–	–	P	P	–	–	–	P	–	–	–	–	P	–	–	–	–
30[c]																				
Sept. 10[d]																				
Oct. 10	+	+	P	–	–	+	+	P	–	–	+	+	P	–	–	+	+	P	–	–
Nov. 4	+	P	–	–	–	+	P	–	–	–	+	+	P	–	–	+	+	P	–	–

[a] Guinea pigs inoculated intra-abdominally with milk from each quarter had typical *Bact. abortus* lesions when autopsies were performed 10 weeks later.
[b] Aborted a 7-month fetus.
[c] Right rear quarter, positive guinea-pig inoculation. Right front quarter lost, and left rear and left front quarters negative.
[d] Aborted a 7-month fetus.

In Table IV is given the record of milk from a cow that has never aborted. On June 16, 1915, agglutinins had appeared in all but the left front quarter. Guinea-pig inoculations made on June 30 were positive for infectious abortion in all but the left front quarter. On October 16, 1915, the reaction had spread to the left front quarter. Milk from this quarter is now being tested by guinea-pig inoculation.

TABLE IV.—*History of milk from a cow that has never aborted*

[Agglutination reaction at middle of milking, when various quantities of milk were added to test tubes containing bacterial suspension]

Date.	Right rear quarter.					Right front quarter.					Left rear quarter.					Left front quarter.				
	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.
1915.																				
Apr. 9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
June 16	+	P	–	–	–	P	P	–	–	–	+	P	–	–	–	–	–	–	–	–
30[a]																				
Oct. 15	+	+	P	–	–	+	+	+	–	–	+	+	–	–	–	+	+	+	+	–

[a] Guinea pigs inoculated intra-abdominally with milk from each quarter had typical *Bact. abortus* lesions and blood reactions, with exception of left front quarter, which was normal.

While, in Tables I to IV, a positive agglutination test points to the presence of *Bact. abortus* in the milk, this is not always true if judged by guinea-pig inoculation. In several cases the writer was unable to get positive lesions in guinea pigs with milk from all four quarters that gave a positive agglutination reaction. In these instances it is probable that the agglutinins were coming from the blood stream, or, if due to a bacterial invasion of the udder, the bacterium may have been present in too small numbers to cause lesions in guinea pigs with the 5 c. c. of milk used for inoculation. In the instances of agglutination with negative guinea-pig inoculation it was noticed that the reaction from quarter to quarter seemed to be fairly constant. In the tables given, the reaction is seen to vary a good deal from quarter to quarter. This, the writer believes, indicated that in the cases of reaction without pathogenicity to guinea pigs the agglutinins were coming to each quarter from a common source the blood.

Though many samples of milk have been inoculated into guinea pigs, at no time has a sample been found with a negative agglutination test that would produce the typical lesions of infectious abortion.

The present value of this test is that it enables one to select from a herd the cows whose udders may be infected with *Bact. abortus*. The comparatively small number separated by this method may then be examined by guinea-pig inoculation and cultural methods.

If *Bact. abortus* is found to be pathogenic for humans, as has been suggested by Melvin,[1] this test may be of value as another means of safeguarding certified and all unpasteurized milk.

From observations and tests now being made it appears that it may be possible to differentiate samples in which the agglutinins come from the blood from those in which the agglutinins are produced in the udder.

SUMMARY.

A pure culture of *Bacterium abortus* Bang introduced into the milk cistern of a cow's udder caused the appearance of agglutinins in the milk.

In every case in which *Bact. abortus* was found present in the milk by animal inoculation the agglutinins for this organism were also found, but this bacterium was not found in every case in which agglutinins were demonstrated.

The agglutination test is of value in studying the presence of *Bact. abortus* in milk when it is desired to study a large number of samples.

If *Bact. abortus* is found to be pathogenic for humans, this test may be of value as another means of safeguarding certified and all unpasteurized milk.

[1] Melvin, A. D. Infectious abortion of cattle and the occurrence of its bacterium in milk. I.—Introductory statement. *In* U. S. Dept. Agr. Bur. Anim. Indus. 28th Ann. Rpt. 1911, p. 137-138. 1913.

BORON: ITS ABSORPTION AND DISTRIBUTION IN PLANTS AND ITS EFFECT ON GROWTH

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INTRODUCTION

The experiments reported in this paper were made in connection with a cooperative study of borax and calcined colemanite as larvicides for the house fly conducted by the Bureaus of Entomology, Chemistry, and Plant Industry, of the Department of Agriculture. The object of this particular study was to determine the effect of boron-treated horse manure on plant growth and to study the absorption of boron and its distribution in the roots, stems, and fruit of plants grown on soil fertilized with this manure and on soil fertilized with untreated manure. The plants were grown in pots in the greenhouses of the Department and on open plots at Arlington Experimental Farm, Va.; Dallas, Tex.; Orlando, Fla.; and New Orleans, La. Analyses of the soil from several treated and untreated plots are included.[1]

Certain deposits of boron have been known for centuries, but the wide distribution of this element in mineral and vegetable matter has been recognized only during the last few years. Probably the first to record the presence of boron in plants were Wittstein and Apoiger (14),[2] who found it in the seeds of *Maessa picta*. Since then many observers have found boron in soils, rocks, fruits, and vegetables.

As soils in many places contain boron, it is not surprising that this element is widely distributed in small amounts in plants. It is also probable that boron is present in nearly all animal material. Bertrand and Agulhon (3) report its presence in the hair, horns, bones, liver, and muscles of animals. They detected boron in 27 species of animals, and state that it probably exists in all animals, being more common in those of marine origin. Boron was also found in human, asses', and cows' milk and in the eggs of the chicken, turkey, and goose.

The toxic effect of boron on plants was first shown in 1876 by Peligot (12), who noted a yellowing of the leaves of beans and reported that in many cases the yellow leaves fell from the plants. The previous year Heckel (8) reported that 1.25 per cent solutions of alkali borate retarded germination for from one to three days, and that 3 per cent of the alkali borate solutions stopped germination entirely. Loew (10, p. 374) states

[1] The writer desires to express his thanks to Mr. W. D. Hunter, of the Bureau of Entomology, for his material assistance in arranging for the experiments in the South.

[2] Reference is made by number to "Literature cited," pp. 889-890.

that certain algæ, such as Spirogyra and Vaucheria, are resistant to the action of boron. Morel (11), however, states that very weak solutions of boric acid arrest the development of lower fungi and similar organisms. He suggests that boric acid may be used, like copper, to attack such diseases as mildew and anthracnose. The effect of boron on the lower plants, fungi, yeasts, etc., has been but little studied.

Agulhon (1) and Bertrand (2) have stated that boron in small amounts acts as a stimulant to plant growth. Pellet (12) calls attention to some experiments which indicate that compounds of both manganese and boron, singly and combined, have no effect on the growth or yield of the sugar beet. He concludes that the results of other workers claiming a stimulation are too few and are untrustworthy.

Many investigations regarding the effect of boron on plants and plant growth have been reported, but no attempt to review all such experiments is made in this paper. For a review of this subject the publication of Haselhoff (7) and the recent work of Brenchley (4), where various inorganic plant poisons and stimulants are discussed, should be consulted.

EXPERIMENTAL WORK

Very few of the previous studies have included a quantitative estimation of the boron present in plants, and no experiments concerning the effects of calcined colemanite (crude calcium borate) on plant growth have been reported. As both borax and calcined colemanite are valuable larvicides for the house-fly maggot, it seems advisable to determine the effect of manure treated with both borax and calcined colemanite on the growth of a variety of plants.

The manure used in these tests was treated with the amounts of borax or calcined colemanite noted in the tables, and stood in the open for 10 days before it was applied to the soil. For the plot tests, the manure was applied at the rate of 20 tons per acre and was then plowed under, the ground harrowed, and sometimes rolled and reharrowed, before planting. In nearly all of these experiments borax or calcined colemanite was applied to the manure in larger quantities than were required to act as a larvicide—i. e., 0.62 per pound per 8 bushels, or 10 cubic feet. When the manure was mixed with the soil at the rate of 20 tons per acre, 216 pounds of borax per acre were present. Furthermore, the manure was not allowed to stand and leach for longer than 10 days; consequently, practically the entire amount of borax added reached the soil.

When 0.62 pound of borax was applied to each 8 bushels of manure and the weight of 8 bushels of manure estimated at 115 pounds (the average weight of fresh manure containing a large amount of straw), 100 pounds of manure contained 0.54 pound of borax, and when the manure was applied to the soil at the rate of 1 part to 40, the percentage of boron in the soil, calculating the weight of 1 acre of soil 6 inches deep as 1,750,000 pounds, was 0.0015.

Tests with tomato (*Lycopersicon esculentum*) and lettuce (*Lactuca sativa*) were made on plants which had been grown in boxes in greenhouses until they were 2 to 3 inches high, when they were transplanted in their respective pots containing the mixtures of manure and soil. The potatoes (*Solanum tuberosum*) tested were of the Green Mountain variety and the seeds used in growing the other plants were common varieties. The percentages of boric acid as recorded in the tables are calculated to a water- and ash-free basis. At least four pots for each treatment were employed in the pot tests. The plots at Arlington Farm were one-twentieth of an acre and those in the South about one-sixtieth of an acre in size. The tests with lettuce were carried out in benches, each 3 by 5 feet.

DESCRIPTION OF METHODS

Many tests for determining boron in foods and other material have been devised. When small amounts are present, as was the case in the present experiments, it is determined colorimetrically, using curcumin, the active principle in turmeric (*Curcuma longa* L.), a characteristic red color being given when boron is present.

In preparing the samples, the roots were separated from the plants. Both roots and plants were washed, dried, and cut into small pieces for analysis. In some cases the fruit also was tested. In such instances it was washed, dried, and ground for analysis. Boron was determined by the use of freshly prepared strips of curcumin paper, prepared by immersing large unfolded filter paper in a 0.2 per cent alcoholic solution of curcumin. The procedure was as follows: About 3 gm. of a dried sample were treated with sufficient saturated lime water to make the reaction alkaline. After a thorough mixing in platinum dishes, the samples were dried and heated in a muffle until all of the organic matter had burned off. Ten c. c. of water and a little hydrochloric acid were added and the solution was warmed, filtered, washed, and made to 100 c. c. volume. A 50 c. c. aliquot was usually taken for the determination of the boron, but this varied according to the amount present. To the 50 c. c. aliquot, or a smaller aliquot diluted to 50 c. c., placed in small porcelain evaporating dishes, 2 c. c. of hydrochloric acid were added, and strips of curcumin paper were suspended and allowed to dip into those solutions to the depth of one-fourth of an inch. In all cases standard boric-acid solutions, as well as blanks, were simultaneously employed. After four hours the colors on the strips of paper were compared and the percentage of boric acid determined.

In the case of soils, the boron soluble in weak hydrochloric acid, not the total boron, was determined. Fifty gm. of soil were shaken with 200 c. c. of a solution of hydrochloric acid (1:20) for one hour. This was filtered and 100 c. c. of the filtrate made alkaline with lime water, evaporated to dryness, and ashed. The ash was acidified with hydrochloric

acid and the solution made to 100 c. c., a 50 c. c. aliquot being used for the colorimetric test. In some cases larger amounts of soil were used for the tests. From 2 to 3 gm. of the plant samples were used for moisture and ash determinations.[1]

RESULTS OF EXPERIMENTS

The results of the experiments are expressed in all the tables and text as percentages of boric acid. Some analyses of boron soluble in weak hydrochloric-acid extracts of soils are also reported. The form of the combination of the boron in plants is not known. The boron of soils is in part present in insoluble combinations with silica, and the absence of acid-soluble boron in some soils may be thus explained. Ash results are also reported for most of the plants analyzed. Separate analyses of the tops, roots, and fruits are tabulated.

In Table I analyses showing the distribution of ash and boron in the tops and roots of wheat (*Triticum* spp.) and beets (*Beta vulgaris*) 3 months old, grown in the presence of calcined colemanite and borax, with and without the addition of lime, are recorded. More boron was found in the tops than in the roots of both plants. The beets absorbed more boron than the wheat plants, especially from the soil treated with calcined colemanite. All of the control plants contained a little boron.

TABLE I.—*Percentage of boron in wheat and beets: Greenhouse pot tests*[a]

Series No.	Treatment of manure per 8 bushels.	Wheat (dry basis).				Beets (dry basis).			
		Tops.		Roots.		Tops.		Roots.	
		Ash.	Boron as boric acid, ash-free basis.	Ash.	Boron as boric acid, ash-free basis.	Ash.	Boron as boric acid, ash-free basis.	Ash.	Boron as boric acid, ash-free basis.
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
1	0.75 pounds of calcined colemanite added	15.55	0.0103	20.00	Trace.	23.10	0.0315	17.74	0.0020
2	1.5 pounds of calcined colemanite added	12.96	.0097	24.76	Trace.	21.69	.0402	12.75	.0025
3	1 pound of borax added	12.58	.0097	33.48	0.0008	22.39	.0120	14.52	.0054
4	1 pound of borax and 1 ounce of lime added	8.51	.0122	23.39	.0029	23.07	.0172	......	.0097
5	1 pound of borax and 3 ounces of lime added	9.63	.0105	25.69	.0044	22.77	.0154	14.12	.0087
6	1 pound of borax and 9 ounces of lime added	11.07	.0173	26.24	Trace.	20.36	.0062	14.41	.0047
7	Control	9.20	.0013	23.76	Trace.	23.80	Trace.	14.56	.0013

[a] Forty parts of soil and 1 part of boron-treated manure were mixed in all the pot and bench tests.

A similar series of tests using tomatoes and cowpeas (*Vigna sinensis*) are recorded in Table II. The number and weight of the tomatoes obtained from four pots, which are also recorded, show the injurious

[1] The analyses were completed with the assistance of Mr. J. B. Wilson, of the Animal Physiological Chemical Laboratory, to whom the writer desires to express his indebtedness.

action of the boron alone and the benefit derived from adding lime. The tops of the tomatoes contained rather a large quantity of boron, the roots and fruit but traces. More boron was absorbed by the tomato plants when borax was added than with the addition of calcined colemanite. The addition of lime with the borax retarded the absorption of boron. The lowest percentage of dry matter was found in the tomatoes grown on the soil where borax alone was added. The tops of the control plants contained the least ash.

TABLE II.—*Boron in tomatoes and cowpeas: Greenhouse pot tests*

Series No.	Treatment of manure per 8 bushels.	Tomatoes.							
		Tops.		Roots.		Fruit.		Yield of fruit.	
		Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).	Dry matter.	Boron as boric acid (ash-free basis).	Number.	Weight.
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per cent.*		*Ounces.*
1	0.75 pound of calcined colemanite added.	13.13	0.0054	9.59	Trace.	6.04	Faint trace.	17	37.25
2	1.5 pounds of calcined colemanite added.	14.44	.0107	10.86	...do...	5.94	.....do.....	16	31.5
3	1 pound of borax added......	11.87	.0146	28.80	None..	4.72	.....do.....	10	10
4	1 pound of borax and 1 ounce of lime added.	12.95	.0123	13.76	...do...	....	....	15	33
5	1 pound of borax and 3 ounces of lime added.	12.15	.0072	10.66	...do...	5.26	Faint trace.	17	34
6	1 pound of borax and 9 ounces of lime added.	12.00	Trace.	19.43	...do...	5.85	.....do.....	18	35
7	Control......................	10.12	...do...	21.88	Trace.	5.92	.....do.....	23	40.25

Series No.	Treatment of manure per 8 bushels.	Cowpeas (dry basis).					
		Tops.		Roots.		Fruit.	
		Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	0.75 pound of calcined colemanite added..........................	9.27	0.0339	18.52	0.0033	3.68	0.0135
2	1.5 pounds of calcined colemanite added..........................	9.25	.0287	27.04	Trace.	3.90	.0106
3	1 pound of borax added...........	8.54	.0242	24.40	None.	3.36	.0133
4	1 pound of borax and 1 ounce of lime added..........................	10.96	.0115	10.01	....do.....	4.12	.0222
5	1 pound of borax and 3 ounces of lime added......................	10.08	.0237	17.44	....do.....	3.01	.0097
6	1 pound of borax and 9 ounces of lime added......................	11.36	.0302	20.64	....	3.40	.0029
7	Control..........................	7.84	.0068	22.58	None.	3.20	.0094

The tops of the cowpeas contained the most boron and the roots the least, the fruit being intermediate. The addition of lime with the borax did not influence the total amount of boron absorbed by the plants. The control cowpeas contained larger amounts of boron than the tomato control plants. The tops of the control cowpeas contained the least ash.

The results of the greenhouse, bench, and pot tests with lettuce and tomatoes are recorded in Table III. It is evident that the lettuce plants took up boron in proportion to the amounts present in the soil. The control lettuce contained the lowest percentage of solids and indicated the presence of boron. A slight chlorosis of the lettuce plants grown in series 1 and 2 was seen, but no injury to the roots was observed. The results of the analyses of the upper and lower 6 inches of soil in the benches show an even distribution of the boron.

TABLE III.—*Boron in lettuce and tomatoes: Greenhouse bench and pot tests*

Series No.	Treatment of manure per 8 bushels.	Lettuce (entire plant).		Soluble boron as boric acid in soil on which lettuce was grown.	
		Dry matter.	Boron as boric acid (dry basis).	Upper 6 inches of soil.	Lower 6 inches of soil.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1	0.75 pound of borax added	11.6	0.00036	0.0012	0.0016
2	1.25 pounds of borax added	10.0	.00064	.0022	.0028
3	Control	9.0	.00020	Faint trace.	Faint trace.
4	0.5 pound of borax added		.00036		
5	0.62 pound of borax added		.00042		
6	0.75 pound of borax added				
7	Control		.00015		

Series No.	Treatment of manure per 8 bushels.	Tomatoes.					
		Tops (dry basis).		Fruit (fresh basis).		Yield.	
		Ash.	Boron as boric acid (ash-free basis).	Dry matter.	Boron as boric acid (water and ash free basis).	Number.	Weight.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*		*Ounces.*
1	0.75 pound of borax added	12.98	0.0089	6.55	Faint trace.	123	157
2	1.25 pounds of borax added	12.94	.0196	6.60	do	101	139¼
3	Control	10.10	.0009	6.75	do	120	159½
4	0.5 pound of borax added	10.01	.015	8.10	0.0002		
5	0.62 pound of borax added	10.77	.016	8.01	.0004		
6	0.75 pound of borax added	7.72		7.51	.0003		
7	Control	7.72	.0015	8.00			

Tomato plants 1, 2, and 3, Table III, were 6 months old at the time of analysis. The yield of fruit from three pots in each series, 1, 2, and 3, showed no reduction in the case of the 0.75-pound borax application, but the 1.25-pound borax application reduced the yield. The dry matter of the control fruit, series 3, is higher than in series 1 or 2, and the ash of the control tops, series 3, is lower than the ash for the tops, series 1 and 2. The tomato plants, series 4, 5, 6, and 7, were younger and smaller than those of series 1, 2, and 3. In all the tomato plants (Table III) the tops contained practically all the boron, the fruit showing only traces.

The results with wheat grown in plots at Arlington Farm, Va., are given in Table IV. The manure was applied at the rate of 20 tons per acre. The wheat was planted in October, 1913, and harvested in June, 1914, the soil samples being tested at the time of harvesting. On the borax plot the wheat plants which were yellow during the winter, became green and normal in appearance in the spring. The yield of wheat from the borax plot was 90 per cent of the control, but larger than that from an unmanured plot which was simultaneously tested. The amount of borax added to the borax plot was about four times that necessary to act as a larvicide, but only a trace of boron was found in the wheat grain or straw. The wheat grains were sound and the nitrogen and ether-extract results of the control differed very little from those of the wheat and straw from the borax-treated plot. A trace of boron was found in the grains and straw from the borax plot, and the borax-treated soil showed 0.003 per cent of boric acid. The soil sample from the borax plot contained more nitrates than the control sample. Nitrogen was estimated by the Kjeldahl-Gunning method, and nitrates by the method recommended by the American Public Health Association.

TABLE IV.—*Percentage of boron in wheat, straw, and soil: Plot tests at Arlington Farm, Va.*

Series No.	Treatment of manure per 8 bushels.	Material.	Nitrogen.	Nitrogen as ammonia (MgO method).	Nitrogen as nitrates.	Ether extract.	Acid-soluble boron as boric acid.
1	2 to 3 pounds of borax added.	Wheat grains	2.15			1.70	Faint trace.
		Wheat straw	.281			2.12	Do.
		Soil 3 to 4 inches deep	.09	0.004	0.0018		0.003.
2	Control	Wheat grains	2.21			1.77	None.
		Wheat straw	.323			2.27	Do.
		Soil 3 to 4 inches deep	.09	.003	.0012		Do.

Results of the analyses of soybeans (*Glycine hispida*), string beans (*Phaseolus vulgaris*), and potato plants grown on plots at Arlington Farm, Va., are recorded in Table V. The roots and beans of the soybeans contained about equal amounts of boron, and rather large quantities were found in the tops of all the plants analyzed. There was a more equal distribution of boron in the roots, tops, and beans of the string beans than in the case of the soybeans.

The potatoes showed only traces of boron in the tops, the largest part of the boron being found in the roots, although the tubers contained a fairly large amount. All control plants contained a little boron. The addition of lime with the borax did not prevent the absorption of boron by the plants, as much boron being absorbed from the calcined-colemanite plots as from the borax plots.

TABLE V.—*Percentage of boron in soybeans, string beans, and potatoes: Plot tests at Arlington Farm, Va.*

Series No.	Treatment of soil per square rod.	Boron as boric acid (dry basis).								
		Soybeans.			String beans.			Potatoes.		
		Roots.	Tops.	Beans.	Roots.	Tops.	Beans.	Roots.	Tops.	Potatoes.
1	1.61 pounds of calcined colemanite added....	0.0086	0.0048	0.0092	0.0044	0.0075	0.0045	0.0170	0.0012	0.0094
2	2.88 pounds of calcined colemanite added....	.0160	.0076	.0136	.0083	.0177	.0213	.0144	Trace.	.0021
3	3.96 pounds of borax added...............	.0124	.0047	.0104	.0086	.0093	.0117	.0354	..do...	.0066
4	3.96 pounds of borax and 2 pounds of lime added...............	.0126	.0040	.0164	.0093	.0099	.0080	.0165	..do...	.0019
5	2 pounds of lime added.	.0030	.0008	.0036	.0050	None.	.0042	Trace.	None.	.0010

In Table VI results of the analyses of corn (*Zea mays*), wheat, peas (*Pisum sativum*), and oats (*Avena sativa*), grown on plots at New Orleans, La., and Dallas, Tex., are recorded. The entire plants, which were 3 months old and small, were used. The corn and wheat plants took up equal amounts of boron. Soluble boron was found in all nine samples of soil from New Orleans, while only two of the five samples from Dallas contained any. The peas absorbed more boron than the oats, especially in series 1, 2, and 3.

TABLE VI.—*Percentage of boron in corn, wheat, peas, oats, and soil: Plot tests at New Orleans, La., and Dallas, Tex.*

Series No.	Treatment of manure per 8 bushels.	New Orleans, La.			Dallas, Tex.		
		Boron as boric acid (entire plant, dry basis).		Soluble boron as boric acid in soil, sample taken 4 inches deep.	Boron as boric acid (entire plant, dry basis).		Soluble boron as boric acid in soil, sample taken 3 inches deep.
		Corn.	Wheat.		Peas.	Oats.	
1	0.5 pound of borax added........................	0.013	0.011	0.0006	0.010	0.001	0
2	0.62 pound of borax added........................	.015	.015	.0009	.010	.006	Trace.
3	Control........................	Trace.	Trace.	Trace.	.003	0	0
4	0.75 pound of borax added........................	....	....	.0003	.040	.025	0
5	1.25 pounds of borax added........................	....	....	.0013	.026	.025	Trace.
6	Control........................	....	....	Trace.	.0005	0	0
7	0.75 pound of calcined colemanite added........	....	....	.0008	....	....	....
8	1.50 pounds of calcined colemanite added........	....	....	.0032	....	....	....
9	Control........................	....	....	Trace.	....	....	....

TABLE VII.—*Percentage of boron and ash in radishes, string beans, cowpeas, peas, and soil: Plot tests at Orlando, Fla.*

Series No.	Treatment of manure per 8 bushels.	Radishes (dry basis).				String beans (dry basis).			
		Tops.		Roots.		Tops.		Roots.	
		Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).
1	0.75 pound of borax added	34.44	0.162	50.08	0.039	....	0.086	22.98	0.011
2	1.25 pounds of borax added	49.49	.226	51.12	.087	17.56	.080	14.89	.015
3	Control	45.25	.018	45.04	.010	22.80	.011	....	.007

Series No.	Treatment of manure per 8 bushels.	Cowpeas (dry basis).				Peas (entire plant, dry basis).	Soluble boron as boric acid found in sample of soil 3 to 4 inches deep.
		Tops.		Roots.			
		Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).	Boron as boric acid.	
1	0.75 pound of borax added	29.49	0.162	35.15	0.222	0.212	0.0006
2	1.25 pounds of borax added	33.22	.140	45.68	.240	.229	.0010
3	Control	20.18	.024	....	.029	.024	.0003

In Table VII the boron content of radish (*Raphanus sativus*), string-bean, cowpea, and pea plants, grown on borax and control plots at Orlando, Fla., is given. An appreciable amount of soluble boron was found in the soil samples from all three plots. The radish plants contained a large amount of boron in the tops, as well as an appreciable quantity in the roots. The string beans did not absorb as much boron as the radishes, but contained a large percentage of the absorbed boron in the tops. The cowpeas absorbed large amounts of boron, more being found in the roots than in the tops. The pea plants also absorbed boron in great quantities. All the control plants contained boron to a marked degree, which is not surprising, as 0.0003 per cent of soluble boron was found in the control soil sample examined at the close of the test.

As there was little rain at Orlando while these tests were being conducted, and as relatively large quantities of soluble boron were found in the samples of soil tested, it is not surprising that the plants absorbed large amounts of boron.

DISCUSSION OF EXPERIMENTAL WORK

It apparently made little difference in the quantities of boron absorbed by the various plants whether it was added to the manure used on the soil in the form of calcined colemanite or as borax. The addition of

lime to the borax also showed no definite action in preventing the absorption of boron, although with beets (Table I) and with one series of tomatoes (Table II) such a reduction is indicated where the largest application of lime was made. Most of the plants analyzed took up boron in proportion to the amounts present in soluble form in the soil.

The leguminous plants, which were most easily injured by boron, absorbed larger amounts than the other plants tested, while wheat and oats absorbed but little boron. It is particularly noteworthy that the wheat grown at Arlington Farm, Va., on soil fertilized with manure heavily treated with boron showed only traces of boron in the grain and straw. Haselhoff (7) found boron in the stalk of maize, but not in the grain.

The most striking differences in the absorption and distribution of boron are shown by the leguminous plants, where a more even distribution between roots, tops, and fruit is found. Potatoes also showed rather a large quantity of boron in the roots and tubers, but only a small amount in the tops. Succulent plants like beets also absorbed boron. On the other hand, tomatoes and wheat showed only traces of boron in the fruit and but little in the roots. Agulhon (1) has investigated the action of boric acid on wheat, using synthetic sterile liquid media, including both soil and water cultures. He recommends 0.0012 per cent of boron to obtain the best growth. In these tests, when borax was added at the rate of 0.62 pound to each 8 bushels of manure and this manure applied to the soil at the rate of 15 tons per acre, 0.0015 per cent of boron was added to the soil.

The fact that all control plants contained a little boron shows the wide distribution of boron in the soil. From the large amounts taken up by the control plants grown at Orlando, Fla., it appears that the soil there contains more than the soil at Dallas, Tex., New Orleans, La., or Arlington Farm, Va.

The ash results of the various portions of the plants analyzed vary considerably, and the variations are not in a definite direction.

A spotting or yellowing of the leaves of plants, which was first noted by Hotter (9) and later reported by several investigators, was observed in these experiments when boron was present in the soil to any extent. In the case of the tomato plants, Table II, a yellowing of the leaves was noted when borax was used at the 0.75-pound rate, but the yield was unaffected. In some of the legumes—namely, string beans, soybeans, and peas—a noticeable yellowing of the leaves was observed when borax was added at the rate of 0.75 pound, and in these cases a reduction in stand took place. The wheat plants grown at Arlington Farm on the plot fertilized with manure treated with from 2 to 3 pounds of borax to each 8 bushels, as noted on page 883, were yellow during the first 3 or 4 months of growth. When the growth started in the spring, however, the plants became green, and the yield of the grain was 90 per cent of the

control yield, more than that obtained from the unmanured control plot. The yellowing of the leaves is an unmistakable sign of injury, although in some cases the plant can recover, or at least is not sufficiently injured to cause a reduction in the yield.

Haselhoff (7) states that the action of boron is more marked on beans than on oats or corn, and that it can be seen when small amounts of boron are present in the soil and when no action injurious to plant growth is evident. He says further that small amounts of boron stimulate the growth of beans and corn, while large amounts produce injury. In his experiments beans absorbed boron in proportion to the amount present in the soil up to a certain limit. The plants examined by Haselhoff contained from 0.04 to 0.17 per cent of boron, which is more than was found in these experiments, with the exception of the plants grown at Orlando, Fla. (Table VII). He suggests that for safety the amount of boron in the soil be less than 0.0001 per cent. According to Brenchley (5), peas are stimulated by relatively high concentrations of boric acid, but with larger applications of boric acid the toxic action was well marked on the leaves, which tend to become brown and to die in a characteristic manner.

There is some evidence in the literature to indicate that small amounts of boron stimulate plant growth. Brenchley (5) states that below a certain dilution boron tends to produce stronger roots and shoots. Large amounts of boron are known to be toxic to practically all plants, with the exception of certain fungi.

In these experiments, where in most cases more boron was added than was necessary to act as a larvicide, no stimulating action was noted. On the contrary, an injurious action was seen with leguminous plants, which became yellow and did not show a good stand. Tomatoes, beets, lettuce, potatoes, radishes, corn, oats, and wheat appeared normal when grown in the presence of amounts of boron which produced injury to leguminous plants. When borax is added to manure at the rate of 0.62 pound to each 8 bushels and the manure is applied to the soil at the rate of 15 tons per acre, 0.0011 per cent of boron is added to the soil. This quantity of boron may injure leguminous plants, but did not injure the other plants tested, although no stimulation was noted. If the borax-treated manure is mixed with untreated manure, as would be done in many cases, since it is necessary to treat manure with borax to destroy fly larvæ during only a portion of the year, it is possible that the percentage of boron would be sufficiently reduced to bring about a stimulating action on plant growth.

In connection with the stimulating action of boron, it may be mentioned that nitrites and nitrates were detected in three or four borax-treated manure piles at New Orleans (6, p. 19), while the corresponding control piles contained no nitrites or nitrates, and several soils fertilized with borax manure have shown more nitrates than the check soils. A

stimulating action of boron on the nitrifying bacteria seems to follow in certain cases.

The results at Orlando, where the same amounts of boron were added to the soil as at other points, but where the toxic action of the boron was marked and where soluble boron was found in the soils after several months, indicates that many factors are involved in the absorption of boron and its effect on plants, and that definite conclusions in studies of this nature should be drawn with great care. These results are submitted as a preliminary study of this question. It is our purpose to test the cumulative action of boron in soils.

SUMMARY

(1) It apparently made little difference in the quantity of boron absorbed by the plants tested whether boron was added to the soil as borax or as calcined colemanite. The addition of lime with borax had no definite effect in preventing the absorption of boron. Wheat and oats absorbed very little boron, while leguminous and succulent plants absorbed comparatively large amounts.

(2) Wheat, beets, cowpeas, and tomatoes grown in pots in the greenhouses contained boron principally in the tops of the plants, and, with the exception of the beets, comparatively little or none in the roots.

(3) The fruit of the tomato plants contained only traces of boron, while the fruit of the cowpea contained large quantities. Lettuce grown in the greenhouse absorbed boron in proportion to the amounts present in the soil.

(4) Potatoes grown in the open showed, when mature, a small amount of boron in the tops and relatively large amounts in the roots and tubers.

(5) The leguminous plants, string beans, soybeans, and cowpeas, which were very sensitive to boron, showed when grown in plot tests a more equal distribution of the boron among the roots, tops, and fruit than the other plants tested.

(6) Radishes grown in plots contained much larger quantities of boron in the tops than in the roots. Analyses of entire plants of wheat, corn, peas, and oats grown on plots in the South showed the absorption of boron in all cases, the peas absorbing the most. All of the control plants contained at least a trace of boron.

(7) Samples of soil from some of the control plots showed the presence of acid-soluble boron, while several similar samples of soil from certain boron-treated plots showed no acid-soluble boron. Usually more soluble boron was found in the treated soil than in the control soil.

(8) The yield of wheat from a plot heavily treated with borax was 90 per cent of the manured-control yield and greater than the yield from the unmanured control. The wheat grains were sound and contained but a trace of boron.

(9) The yield of tomatoes in pot tests was unaffected when borax was added in amounts to produce 0.0018 per cent of boron in the soil, but when the amount was increased to 0.0030 per cent, a reduced yield resulted.

(10) Numerous factors influence the absorption, distribution, and action of boron in plants.

(11) No more than 0.62 pound of borax or 0.75 pound of calcined colemanite should be added to each 10 cubic feet of manure, and when using the boron-treated manure in growing leguminous plants, the manure should be mixed with untreated manure before being applied to the soil. For other plants, boron-treated manure should not be used at a higher rate than 15 tons per acre.

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FURTHER STUDIES ON PEANUT LEAFSPOT

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INTRODUCTION[1]

A report of investigations of certain fungous diseases of peanuts has previously[2] been made. Since the appearance of that report the investigations have been continued for the purpose of obtaining additional data on certain phases of the work. Opportunity had not been afforded prior to the present year to test under field conditions the efficacy of rotation and seed treatment in the control of leafspot, *Cercospora personata* (B. and C.) Ellis. Definite experimental data upon the agencies concerned in the distribution of leafspot had not been secured; neither had an effort been made to definitely correlate the destructiveness of the disease with the presence of certain climatic conditions. It was the primary purpose of the present work to secure information upon these phases of the subject. The results of these studies are, therefore, recorded as additions to the information contained in the previous publication[3] upon investigations which were begun four year ago under the Adams fund.

ROTATION TESTS FOR LEAFSPOT CONTROL

Because of the fact that the leafspot organism was found to live on fallen leaves in the field from one season to the next,[4] it was recommended as a rational method of control that the same fields be not planted to peanuts in successive years. Observations on the effectiveness of rotation were made at several widely separated points in the State, with the representative results which are recorded in Table I.

In many cases it has been difficult to get reliable information as to the crops previously grown upon the fields in which these studies were made, since the tenants knew nothing of the system of cropping employed prior to their tenure. In determining the percentage of plants affected, the

[1] The writer received valuable aid in the field tests from R. C. Lett, farm adviser for Tuscaloosa County, Ala., on whose farm the seed-treatment tests were conducted, and from S. A. Wingard, who carefully and arduously assisted in the field studies. Indebtedness is hereby acknowledged to both gentlemen for these several services.

[2] Wolf, F. A. Leafspot and some fruit rots of peanut. Ala. Agr. Exp. Sta. Bul. 180, p. 127–150, 5 pl. 1914. Bibliography, p. 148–149.

[3] Wolf, F. A. Op. cit.

[4] When these leaves [diseased leaves which had remained out of doors from November until May] were kept moist as when placed in moist chambers, conidia were abjointed. Additional evidence that the fungus remains viable is to be found in the fact that leaf spots developed during May, on young plants, in a field which had grown a badly diseased crop the previous season. (Wolf, F. A. Op. cit., p. 135.)

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total number of plants on a certain area was first counted, and then a count was made of those plants which were diseased. A plant having only a single spot on one of its leaves was regarded as diseased. Several attempts were made to determine the decrease in yield due to leafspot, but no satisfactory method has been found and the figures given are only approximate, since they were obtained by determining the average difference in the number of peas borne on 10 healthy and 10 diseased bunches having apparently the same-sized tops. It will be noted that the percentage of diseased plants in fields designated as 1 to 7, which are representative of rotations, varies from 13.5 to 100 per cent. When the results for the fields numbered 4 and 8 are contrasted, the former having borne no peanuts previously for 11 years and the latter having grown four successive crops, with 95 and 100 per cent, respectively, of the plants diseased, with practically no difference in the severity of attack, one is forced to conclude that rotation in itself is not to be regarded as a control measure against peanut leafspot. These results came somewhat as a surprise to the writer. Several reasons for the inefficacy of rotation as a means of leafspot control will be brought out later in this report. It might be suggested at this point, however, that this much overworked and overrecommended suggestion for the control of plant maladies is not a panacea, but requires experimental proof for each particular trouble for which it is recommended.

TABLE I.—*Summary of rotation tests with peanuts made in Alabama in 1915*

Field No.	Location.	Previous crops on soil.	Date of examination.	Plants affected.	Decrease in yield of peas.
				Per cent.	*Per cent.*
1	Auburn	Peanuts had been grown 2 years before	Sept. 6	100	(a)
2	Eutaw	Peanuts had not been grown for several years previously.	Aug. 28	54	5
3	...do	Peanuts had not been grown for 4 or 5 years previously.	30	41	4.5
4	...do	No peanuts had been planted for at least 11 years	31	95	19.5
5	...do	No peanuts had been planted the previous year; no previous record available.	Sept. 1	26	(a)
6	do	No peanuts in field the previous year	1	100	(a)
7	...do	No peanuts in field for 4 years previously	1	13.5	(b)
8	...do	Peanuts had been grown during each of the 4 preceding years.	Aug. 27	100	20

a Not estimated. b Negligible.

SEED DISINFECTION FOR LEAFSPOT CONTROL

Seed treatment for the control of leafspot was recommended in a previous report [1] for two reasons. It had been found that conidia adhere to the surface of the shells, and it had been noted repeatedly that the disease occurs in fields not previously planted to peanuts. It was suggested that solutions of copper sulphate or formaldehyde be used in dis-

[1] Wolf, F. A. Op. cit., p. 134. "The prevalence of leaf spot in lands not previously cultivated is not uncommon . . . conidia and conidiophores have been found in the centrifuged washings of peas."

infecting. In case the former was employed, the peas were to be immersed for 15 minutes in a solution containing 1 pound of copper sulphate to 20 gallons of water; in case the latter was used, 1 pint of formaldehyde to 20 gallons of water, the peas to be steeped for an hour. Tests of the effectiveness of these seed treatments were made during the past season (1915) at Eutaw, Ala. One field, designated as field 10, had previously grown several successive crops of peanuts; the other, field 11, had not been cropped with peanuts at least during the four preceding years. Each field was divided into four plots. Plot 1 in each field was planted with unshelled peanuts which had been immersed in copper sulphate; those in plot 2 were not shelled and were immersed in formaldehyde; those in plot 3 were given no treatment; in plot 4 no fungicide was employed, and the peanuts were shelled prior to planting. The conditions in field 10, as noted in three successive examinations, are given in Table II.

TABLE II.—*Summary of results of leafspot tests on field 10 in 1915, infested with leafspot*

Plot and treatment.	Aug. 6.	Aug. 14.	Aug. 21.
Plot 1. Peanuts, not shelled, steeped in copper sulphate:			
Total number of diseased leaves on 25 plants	882	1,492	3,201
Number of diseased leaves per plant—			
Maximum	79	110	273
Minimum	10	11	23
Plot 2. Peanuts, not shelled, steeped in formaldehyde:			
Total number of diseased leaves on 25 plants	755	1,437	3,020
Number of diseased leaves per plant—			
Maximum	63	144	241
Minimum	3	5	29
Plot 3. Peanuts, not shelled, no treatment:			
Total number of diseased leaves on 25 plants	1,022	2,028	4,234
Number of diseased leaves per plant—			
Maximum	94	178	297
Minimum	7	12	39
Plot 4. Peanuts, shelled, no treatment:			
Total number of diseased leaves on 25 plants	545	1,184	2,749
Number of diseased leaves per plant—			
Maximum	45	128	204
Minimum	2	15	26

Infections were observed in this field as early as July 26 and were probably present at a much earlier date. Plot 3 will be seen to have had a larger number of diseased leaves on August 6, and during the two successive weeks, than did any of the other plots. It would naturally follow from this that seed disinfection is not without appreciable effect. It was felt, however, that it would be necessary to duplicate these results in several localities during several seasons before one could safely conclude that seed treatment is of any practical value, especially in the light of the data to be subsequently presented.

Field 11, which can be directly contrasted with field 10, really shows the result of seed treatment coupled with rotation. No tabulation for field 11, such as has been made for field 10, has been prepared, but the important facts in regard to this field are as follows: Leafspot was not apparent until August 6, 11 days after it was first seen in field 10. Only five plants in the whole field were found to be affected on this date, and

the disease was evidenced by only one or two spots on each leaf. Two of these plants were found in plot 1, one in plot 2, and two in plot 4. It will be noted that on this date plot 1 of field 10 had a maximum of 79 affected leaves on a single plant, plot 2 had 30, plot 3 had 94, and plot 4 had 45. A final count of the number of diseased leaves in field 11 was made on September 1, with the result that 12 per cent of the leaves in plot 1 were affected, 11 per cent in plot 2, 15 per cent in plot 3, and 14 per cent in plot 4. It should be said in explanation that none of these plants had more than six affected leaves, and most of them had only one or two, upon which there were at most only a few spots. Ten days prior to this a final count on field 10 showed a minimum of 23 diseased leaves per plant and a maximum of 297. This number would, no doubt, have been considerably greater by September 1.

The most significant conclusion that one is forced to make from these tests is that seed treatment, either by itself or in conjunction with rotation, does not eliminate peanut leafspot. This conclusion is further supported by the results obtained from the rotation tests given in Table I. The peas used in planting fields 1, 2, 5, 6, and 7 were shelled prior to planting, thus eliminating the danger of introducing infective material at the time of planting. In these fields, 100, 54, 26, 100, and 13.5 per cent, respectively, of the plants were affected with leafspot. The peas used in planting fields 3 and 4 were not shelled, and 41 and 95 per cent, respectively, of the plants were diseased. As can readily be seen from these figures, the removal of the shells prior to planting contributed nothing toward keeping the crop free from disease.

DAMAGE SUSTAINED BY PEANUT PLANTS AS A RESULT OF LEAFSPOT

In order to measure the degree to which leafspot affects the foliage of peanuts, an effort was made to determine the relation between the total leaf area and the diseased area of a peanut plant. The plant used was taken from field 10 and may be regarded as a plant having an average proportion of diseased tissues. The method employed consisted in weighing pieces of paper corresponding in area to the total and the diseased leaf surface. From paper of good quality, pieces, each equal in area to one of the leaves of the plant, were cut. After these had been weighed, areas corresponding to the diseased parts of the leaves were outlined, and these areas were then removed. The paper leaf areas with the excised diseased areas were again weighed with the following computed results: The total weight of the leaves on a single plant is found to be 64.07 gm. Of this weight, 20.10 gm. are wholly free from spots; 12.39 gm. are dead as a result of the attacks of *Cercospora personata* and have for the most part fallen off; the remainder, 31.58 gm., are regarded as diseased leaves. Of these diseased leaves 10.18 gm., or 32.04 per cent, are occupied by the fungus. When 12.39 gm. and 10.12 gm. are combined, it is found that 35.07 per cent of the entire leaf area is lost to photosynthetic activity. It

is realized, of course, that these figures represent only an approximation, because the method itself is inexact. It is believed, however, that the approximated losses in yields of from 5 to 20 per cent given in Table I are reasonable, when one considers that there has been a loss to the plant of about 35 per cent in its active leaf area.

TESTS ON DISSEMINATION OF LEAFSPOT BY AIR AND WIND

Previous work on air currents as an agency in the dispersal of the leafspot fungus yielded only negative results.[1] It was believed, however, in spite of this negative evidence, that conidia are carried short distances by the wind.

The purpose of the tests herein reported was not only to determine whether or not the wind acts as an agent in dissemination of the conidia of *Cercospora personatæ*, but also to ascertain the conditions of temperature and humidity which might influence its maximum or minimum prevalence in the air. The tests were conducted at Eutaw and Auburn, Ala. The tests at Eutaw, Ala., at which place 210 exposures of plates were made, covered the entire period, nights as well as days, from August 9 to August 26, with the exception of August 15 and August 22. The tests at Auburn, Ala., were conducted from September 6 to September 11 and were made to substantiate the tests made at Eutaw, Ala.

The method formerly employed consisted in the exposure for varying lengths of time of sterile agar in Petri dishes. This method is open to objection for the reasons that at certain times the conidia of *Cercospora personata* germinate poorly or not at all and the development of colonies proceeds so slowly that they are likely to be obscured by more rapidly developing forms. It was decided, therefore, to use essentially the method employed by Burrill and Barrett[2] in their study of the dispersal of *Diplodia zeae*. Stations 2, 4, 6, and 8 feet distant from the nearest peanut plant were established. A frame to hold the exposure plates in a vertical position about 8 inches from the ground was made. This frame could be moved at the beginning of each exposure, to permit the plates to face toward the prevailing wind. Glass plates 4 by 5 inches were smeared with glycerin only on the side directed toward the peanut plants. Four sets of exposures of three hours duration each were made during the period from 6 a. m. to 6 p. m. One set of exposures of 12 hours duration was made nightly from 6 p. m. to 6 a. m. Rains interfered somewhat with this routine. Plates exposed during a rain were washed off, and those exposed in the periods following rain were found to be free from conidia. Readings of the temperature and relative humidity were made at the beginning of each set of exposures. The

[1] "All attempts to gain definite data showing that the wind is a carrier of the conidia have thus far been unsuccessful." (Wolf, F. A. Leafspot and some fruit rots of peanut. Ala. Agr. Exp. Sta. Bul. 180, p. 134. 1914.)

[2] Burrill, T. J., and Barrett, J. T. Ear rots of corn. Ill. Agr. Exp. Sta. Bul. 133, p. 63-109, 11 pl. 1909.

exposed plates were brought into the laboratory as soon as possible after collection, were placed edgewise in a glass funnel, and the glycerin and contents washed off into a vial with a 2 c. c. pipetteful of 95 per cent alcohol. The stream of alcohol used in washing the plates was permitted to play slowly along the upper edge. The washings were then permitted to evaporate until only a few drops remained in the vials. By examination with the low-power lens of a microscope the number of conidia in these few drops could then be determined.

This method is open to two serious objections. Many of the spores were not washed from the plate by this method, as evidenced by a test in which a plate washed according to the method described and found to have entrapped three conidia of *Cercospora personata* was afterwards washed, using a wash bottle as a means of driving a stream of 95 per cent alcohol forcibly against it, and was found to have nine additional conidia. The other objection, which was encountered by Heald, Gardner, and Studhalter,[1] consists in the fact that it is practically impossible to spread a film of glycerin uniformly on a glass slide and have it remain so for three hours. The results shown in Table III are therefore not representative of the number of conidia that were actually entrapped, but convincingly prove that the conidia of *C. personata* are wind borne.

TABLE III.—*Results of tests of glycerin plates exposed to air currents at Eutaw, Ala.*

Date of exposure.	Number of plates exposed.		Number of plates with adhering conidia.		Rainfall.	Maximum number of conidia of *Cercospora personata* on any plate.	Total number of conidia entrapped during the entire period.	
	Day.	Night.	Day.	Night.			Day.	Night.
					Inches.			
Aug. 9	12	3	5	1	0	4	15	1
10	12	3	4	0	1.58	3	10	0
11	12	3	5	0	0	4	11	0
12	12	3	5	1	0.43	4	15	1
13	12	3	10	0	0	4	24	0
14	12	3	8	1	0.18	4	16	1
16	12	3	2	0	2.00	2	3	0
17	12	3	2	0	0.37	1	2	0
18	12	3	6	1	0	3	9	1
19	12	3	5	0	0.04	4	12	0
20	8	2	2	0	0.13	3	4	0
21	8	2	4	0	0	3	7	0
23	8	2	3	0	0.02	2	5	0
24	8	2	3	1	1.12	3	6	1
25	8	2	3	1	0	3	5	1
26	8	2	5	0	0	2	6	0
Total	168	42	72	6	....	....	150	9
Total day and night	210		78		....	....	159	

It is not deemed necessary to give a detailed daily record of the actual routine pursued. It will be seen that only 78 of the 210 plates exposed

[1] Heald, F. D., Gardner, M. W., and Studhalter, R. A. Air and wind dissemination of ascospores of the chestnut blight fungus. *In* Jour. Agr. Research, v. 3, no. 6, p. 493-526, pl. 63-65. 1915. Literature cited, p. 525-526.

were found to have adhering conidia. The usual number found was three or four on each plate. The occurrence of rain and heavy dews will in part account for the relatively small number of plates upon which conidia were found. Rain fell on 9 of the 16 days during which these tests were made. The plates washed off by these rains numbered 26. Three sets of exposures of three plates each remained free from conidia in the periods immediately following rain. In many cases one plate only of each set gave positive evidence in the period following. Only six out of the 42 plates exposed at night yielded any positive results, owing principally to the occurrence of dews.

At no time during the period in which these tests were made, as will be seen, was there a maximum period of spore dispersal. Conidia were present in the air, except where it had been rendered free from them by precipitation, during the entire period. This is in accord with the increase in amount of leafspot shown in the successive counts made in field 10 and recorded in Table II. There was approximately twice as much leafspot in field 10 on August 14 as on August 6, and twice as much on August 21 as on August 14. No correlation between these increases and the temperature and humidity records could be discovered, and these figures have consequently been omitted from Table III. The idea formerly entertained[1] that the occurrence of peanut leafspot is correlated with certain moisture and temperature conditions is now regarded as without foundation. Such a correlation would be meaningless in view of the positive evidence, next to be reported, that insects act as carriers of leafspot. Details of the tests conducted at Auburn, Ala., are not tabulated, since the work accords with the work done at Eutaw, Ala., and substantiates the significant fact that air currents are agents in the dissemination of *Cercospora personata.*

INSECTS AS AGENTS IN DISSEMINATION OF THE LEAFSPOT ORGANISM

The fact that the leafspot fungus is air-borne explains in part at least the failure to secure perfect control in the tests in which rotation and seed treatment were combined. No tests have been made, however, upon the distance which the conidia may be transported by the wind. The most distant exposures were only 8 feet from the nearest diseased plant. It seems unlikely that air dispersal could account for severe infection in fields in which both rotation and seed treatment had been practiced and which were from ¼ to ½ mile distant from the nearest infected field. It was therefore suspected that certain insects, among which grasshoppers are the most important, are agents in this spread of leafspot.

[1] "Apparently infection with Cercospora is in some manner correlated with certain moisture and temperature conditions. . . The ravages of *Cercospora personata* seem to attain their maximum severity after a dry period followed by excessively sultry weather. . ." (Wolf, F. A. Leafspot and some fruit rots of peanut. Ala. Agr. Exp. Sta. Bul. 180, p. 133. 1914.)

A relatively meager literature dealing with the subject of insects as carriers of fungi producing plant diseases has accumulated. Since the most important publications upon this subject are summarized in a recent excellent paper by Studhalter and Ruggles,[1] an historical review is purposely omitted at this time. These authors find that certain insects belonging to the orders Hemiptera, Coleoptera, Diptera, and Hymenoptera are carriers of the chestnut-blight organism. Because of the positive evidence secured in the few studies previously made on insects as agencies in the dissemination of plant diseases, it will not be surprising if it is found in future investigations that insects are a very important factor in the dispersal of many plant-pathogenic fungi.

The insects used in these tests were collected in diseased peanut fields near Eutaw, Thomasville, Marion Junction, Greensboro, and Auburn, Ala., and placed in sterile test tubes or flasks plugged with cotton. After being brought into the laboratory, each insect was dropped into a measured amount of water, in case it was desired to determine the number of conidia upon its body. After agitating the tubes vigorously a drop of the wash water was examined under the low-power lens of a microscope, the number of conidia in the drop were counted, and from this the total number of conidia was estimated.

In case fecal discharges were examined, each deposit was macerated in a drop of water on an object slide, and a count was made with the aid of the low-power lens. Because of the presence of undigested bits of plant tissue and the impossibility of one's being sure that no conidia escaped notice and that none were unwittingly counted twice, these determinations can not be exact. They very closely approximate the true number, however, since several counts of the same slide were made and the average taken as the final number.

A total of 75 insects collected in five different counties has been examined in the course of these tests, 54 of which gave positive results. Four orders of insects—namely, Orthoptera, Lepidoptera, Coleoptera, and Hemiptera—were represented among the positive tests. Of the 56 grasshoppers and katydids examined, 38 were found to be bearers of *Cercospora personata*. No attempt has been made to classify these Orthoptera, but several different genera were represented. Of the roasting-ear worms, *Heliothis obsoleta*, which were examined, nine were found to void conidia of Cercospora in their feces. Eight members of the Coleoptera were examined, six of which gave positive results. Three of these were lady beetles, *Megilla maculata;* one a blister beetle, *Epicauta vittata;* and the other two were fireflies, *Chauliognathus* sp. A single member of the Hemiptera, one of the leaf hoppers, was examined and found to be a carrier.

[1] Studhalter, R. A., and Ruggles, A. G. Insects as carriers of the chestnut blight fungus. Penn. Dept. Forestry Bul. 12, 33 p., 4 pl. 1915.

Table IV records the results of an examination of 36 of the 75 insects collected. The remainder of the record is not given, since it would add nothing which is not indicated in this tabulated portion.

TABLE IV.—*Record of examination of insects for conidia of Cercospora personata*

No.	Name of insect.	Date of collection.	Locality.	Number of conidia of *Cercospora personata*. On body.	Number of conidia of *Cercospora personata*. In feces.	Other fungi.	Remarks.
1	Grasshopper	Aug. 10	Eutaw, Ala.	1	8		
2	do	14	do	1			No note was made of the occurrence of *Cercospora personata* or other organism in feces.
3	Roasting-ear worm (*Heliothis obsoleta*).	14	do	2			
4	Grasshopper	16	do	4			
5	do	16	do	1			
6	do	16	do	5			
7	do	26	do	4			
8	do	26	do	6			
9	do	26	do	3			
10	Firefly (*Chauliognathus* sp).	26	do	3			
11	Grasshopper	Sept. 6	Auburn, Ala.		1,250	Species of Alternaria, Helminthosporium, and Fusarium.	Seven insects were placed in a flask and within a half hour after their capture they were examined. By agitating them in 25 c. c. of water conidia from the surface of the bodies and from the feces are included.
12	do	7	do	0	0	2,500 conidia of *Helminthosporium Ravenelii*.	
13	do	8	do	10	8	Fusarium, Alternaria.	
14	do	8	do	0	13	Species of Fusarium and Alternaria.	
15	do	8	do	0	0		
16	do	9	do	0	18		No determination of conidia on bodies was made.
17	do	9	do	0	250		
18	do	9	do	0	200		Single fecal discharge.
19	Roasting-ear worm (*Heliothis obsoleta*.)	9	do	0	1,050		
20	Lady beetle (*Megilla maculata*).	9	do	8	0		
21	Roasting-ear worm (*Heliothis obsoleta*).	10	do	0	50		Total number of conidia contained in three fecal discharges. The presence of conidia upon the bodies was not determined.
22	do	10	do	0	17		
23	do	10	do	0	39		
24	do	10	do	0	27		
25	do	10	do	0	0		
26	do	10	do	0	17		
27	do	10	do	0	28		
28	do	10	do	0	25		
29	Blister beetle (*Epicauta vittata*.	10	do	0	27		Two fecal discharges were examined.

TABLE IV.—*Record of examination of insects for conidia of Cercospora personata*—Continued

No.	Name of insect.	Rate of collection.	Locality.	Number of conidia of *Cercospora personata*. On body.	Number of conidia of *Cercospora personata*. In feces.	Other fungi.	Remarks.
30	Lady beetle (*Megilla maculata*).	Sept. 10	Auburn, Ala.	9	0	....	
31	Grasshopper.......	18	.....do......	0	6	Several hundred conidia of *Helminthosporium Ravenelii* present.	
32	Katydid...........	18	.....do......	0	92	....	Two discharges.
33	Grasshopper.......	18	.....do......	0	3	Many *Fusarium* sp. conidia.	
34	.....do............	20	.....do......	0	6	Few *Alternaria* sp. conidia.	
35	.....do............	20	.....do......	0	0	*Puccinia cassipes* B. and C., *Helminthosporium Ravenelii*.	Over 500 spores of each estimated to be present in a single discharge.
36	Leaf hopper.......	20	.....do......	8	0	....	

Grasshoppers were found to carry *Cercospora personata* conidia on their bodies and also to void them in their feces. The number of conidia to be found within and upon any individual insect depends naturally upon whether or not it has eaten diseased tissue within a short time prior to its capture. The largest number of conidia of *C. personata* found in a single fecal discharge of a grasshopper brought in from the field was 250.

In order to ascertain whether or not feeding grasshoppers either avoid or select diseased leaf tissue, 13 were brought into the laboratory, where they could be closely observed and given diseased peanut leaves as food. Three of them seemed to prefer leafspot tissue, since they ate little except the affected tissue. The others were indifferent in their choice of food, but seemed not to avoid the diseased spots. The conidia in the discharges of some of these insects were too numerous to count.

Passage through the alimentary canal of grasshoppers does not destroy the power of germination of the conidia of *Cercospora personata*. Conidia which had been voided were found to germinate within 12 to 18 hours when placed in drops of water. In fact, some were found to have already germinated at the time of discharge. When it is realized that these conidia-laden discharges are suitable situations for spore germination and a favorable pabulum for subsequent growth, and that they are commonly deposited upon leaves, it is seen that this is not an impossible means of causing infection. Since grasshoppers, which have notoriously strong powers of flight, were among the insects examined with positive results, they no doubt are potent agencies in the dissemination of leafspot for considerable distances. It is believed that the peculiar results in the tests on rotation and seed disinfection, as well as the correlation between the presence of leafspot and certain temperature and moisture conditions

previously reported, is due in part to the fact that grasshoppers and certain other insects are carriers of the leafspot organism.

It might be interesting to note in this connection that it seems to be generally true that peanut fields in which grass and weeds had been permitted to grow unmolested, as exemplified by fields 4 and 6 in Table I, and which consequently afforded a more attractive feeding ground for grasshoppers, are much more severely attacked by Cercospora than those in which good cultivation had been given. Several small fields have also been found upon which chickens and turkeys ranged in which leafspot was doing inappreciable harm, while fields somewhat farther away from the farm buildings were seriously affected. It is believed that the relative freedom from leafspot here observed is to be attributed largely to the destruction of the insects by fowls.

In most cases no attempt was made to determine the presence of other fungi upon the insects taken. Among the other forms noted, however, were *Helminthosporium Ravenelii* B. and C., an organism very abundant upon the inflorescence of *Sporobolus indicus; Puccinia cassipes* B. and C., which is parasitic on species of Ipomoea, a common weed; and species of Alternaria and Fusarium. According to an estimate made, a single fecal deposit of a grasshopper contained 2,500 conidia of *Helminthosporium Ravenelii.* A katydid taken at Marion Junction and one at Auburn each voided a vast number of morning-glory rust spores. Insects 21 to 28 (Table IV) indicate the manner in which this form may carry infections for short distances. The blister beetle is another insect which feeds upon peanut plants and which therefore discharges conidia from its alimentary canal. The other species of insects taken appear to carry conidia only upon their bodies. It seems very probable, judging from the evidence at hand, that any insect which feeds upon peanut foliage is a disseminator of leafspot, and that any of them which frequent peanut fields may serve as carriers.

SUMMARY

(1) Rotation by itself is not effective under field conditions in eliminating leafspot, as evidenced by a field in which peanuts had not been grown for 11 years and in which 95 per cent of the plants were diseased by August 31, with an estimated loss in yield of 19.5 per cent.

(2) Seed disinfection with copper sulphate or formaldehyde before planting does not prevent leafspot. Shelling peanuts before planting to eliminate the danger of infection from conidia which may have been adhering to the surface of the shell does not prevent the disease. Seed treated in these ways, when planted on land which had previously borne diseased peanuts, produced a crop which was 100 per cent diseased. Seed treated and planted on soil which had borne no peanuts for at least four years previously produced a crop 13 per cent of whose plants were more or less affected with leafspot. Crop rotation, therefore, when combined with seed treatment, will not eliminate leafspot.

(3) An approximation of the total leafspot area involved by *Cercospora personata* showed that the photosynthetic area had been decreased 35.07 per cent. Estimations of decrease in yield of peas of from 5 to 20 per cent as the result of leafspot are therefore regarded as reasonable.

(4) No correlation between the presence of certain conditions of temperature and moisture and the prevalence of leafspot exists, because of the fact that air currents and certain insects are carriers of *Cercospora personata.*

(5) As the result of 210 glycerin exposure-plate tests at Eutaw, Ala., substantiated by a series at Auburn, Ala., it is concluded that *Cercospora personata* is wind borne. Seventy-eight of these 210 exposure plates gave positive results. At no time from August 9 to August 26 was there a period of maximum spore dispersal as revealed by the exposure plates. The maximum number of conidia entrapped on any single plate was four. This does not represent the true condition, since the method used in washing the plates failed to remove all conidia. Rains rendered the air temporarily free from Cercospora, and dew prevented the dispersal of conidia at night and in the early morning.

(6) From an examination of 75 insects collected in five localities, of which 54 gave positive results, it is concluded that insects are disseminators of the leafspot fungus. Four orders of insects are included in these positive tests: Orthoptera, represented by grasshoppers and katydids; Lepidoptera, by larvæ of *Heliothis obsoleta;* Coleoptera, by lady beetles, blister beetles, and fireflies; and Hemiptera, by leaf hoppers. Grasshoppers, katydids, roasting-ear worms, and blister beetles eat diseased peanut foliage and void conidia in their fecal discharges. A single deposit from a grasshopper contained 250 conidia of *Cercospora personata.* Another specimen discharged 2,500 conidia of *Helminthosporium Ravenelii* in a single deposit. Grasshoppers may also carry conidia on the surface of their bodies. Leaf hoppers, lady beetles, and fireflies transport conidia on their bodies as a result of having come in contact with diseased leaves. A larva of *Heliothis obsoleta* voided a maximum of 1,050 conidia of *Cercospora personata.* Other fungi, among which are *Puccinia cassipes, Alternaria* sp., and *Fusarium* sp., were found in the fecal discharges of grasshoppers and katydids.

(7) Alimentation in insects does not destroy the viability of *Cercospora personata.*

(8) Grasshoppers, because of their powers of flight, are capable of carrying the leafspot organism considerable distances. The ineffectiveness of crop rotation combined with seed treatment to eliminate leafspot from peanut fields is very probably due to the fact that air currents and certain insects are agents in its dissemination.

RELATION BETWEEN THE PROPERTIES OF HARDNESS AND TOUGHNESS OF ROAD-BUILDING ROCK

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It has for some time past become increasingly evident to engineers interested in the testing of road materials that from the standpoint of the road builder some of the most important physical properties of rock are not independent, but are more or less definitely related to each other. In 1913, Mr. L. W. Page,[1] Director of the United States Office of Public Roads and Rural Engineering, called attention to some of these points, and suggested that, as the volume of data relating to the subject became greater, it might be possible to determine the dependent variable by reference to suitable curves showing the relative values of tests for thousands of individual cases, and thus dispense with one or more of the tests now in use. The large amount of additional data which have accumulated since that time makes it possible to take up the subject again, with a view to determining just what physical tests are necessary in order to judge properly the fitness of a rock for use in road construction.

It is now generally recognized that any stone, to be suitable for use in macadam construction, must possess to a certain degree, depending on circumstances such as character of traffic and method of construction, three distinct physical properties, which may be briefly defined as follows:

(1) Hardness, the resistance which a rock offers to the displacement of its surface particles by abrasion;

(2) Toughness, the resistance which a rock offers to fracture under impact;

(3) Binding power, the ability which the dust from the rock possesses, or develops by contact with water, of binding the larger rock fragments together.

Of these, the first two are of particular interest from the standpoint of the present discussion, and they may be very briefly described as follows:

The degree of hardness of a rock is determined by what is known as the Dorry method. It consists essentially of subjecting a cylinder, 25 mm. in diameter, of the material to be tested, to the abrasive action of crushed quartz sand fed upon a revolving steel disk, against which the test

[1] Page, L. W. Relation between the tests for the wearing qualities of road-building rocks. *In* Amer. Soc. Testing Materials, Proc. 16th Ann. Meeting, 1913, v. 13, p. 983-992, 7 fig., 1913. Discussion, p. 993-995.

—— Tests of materials used in the construction of macadamised roads. Permanent Internat. Assoc. Road Cong., 3d Cong. London, 1913, Rpt. 76, 27 p., 15 fig. 1913.

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specimen rests. The end of the specimen is ground away in inverse ratio to its hardness, so that the hardness may be computed by determining the loss in weight after any given number of revolutions of the disk. The coefficient of hardness discussed later is obtained by subtracting one-third of the loss in weight in grams from 20, after 1,000 revolutions of the disk.

The degree of toughness is determined by the Page impact method. A cylinder 1 inch in diameter and 1 inch high, cut from the rock specimen, is subjected to the impact caused by the free fall of a 2-kgm. weight dropped from successively increasing heights until the energy of the blow is sufficient to fracture the test specimen. The test consists of a 1-cm. fall for the first blow, followed by falls increased by 1 cm. after each blow until failure occurs. The height from which the weight drops when failure takes place is used as a measure of the toughness of the material.

Since the establishment of the Road-Material Laboratory by the United States Government, upwards of 3,000 samples, representing every known variety of road-building rock, and obtained from every State in the Union, as well as from foreign countries, have been subjected to the tests outlined above. The results of these tests are plotted in graphic form in figure 1. The coefficients of hardness are plotted as abscissæ and the factors of toughness as ordinates. Each small circle represents the corresponding hardness and toughness of an individual rock sample. The large circles represent the average of all the coefficients of hardness for each value of toughness. Hardness values range from 0 to 20 and toughness values from 1 to 47.

A study of this curve brings out the following points:

(1) That the average toughness for all tests made is about 9.

(2) That the average hardness increases with toughness, and that the rate of increase becomes less as the toughness values become larger.

(3) That individual values of hardness vary through wide limits for low values of toughness, and that the variations from the average decrease uniformly with the increase in toughness up to a certain point, about 20, after which they remain constant with very little variation from the average.

(4) That, when any given value for toughness falls within certain limits, which define the suitability of the material for macadam-road construction under different traffic conditions, the corresponding value for hardness will fall within similar limits for hardness.

The first three facts are clearly indicated, but in order to substantiate the last deduction it will be necessary to define the limiting values of hardness and toughness which experience has shown should be applied when judging the fitness of stone for use in macadam construction under different traffic conditions. Such limiting values for toughness are shown on the curve in the ordinates at 4.5, 9.5, and 18.5, and the

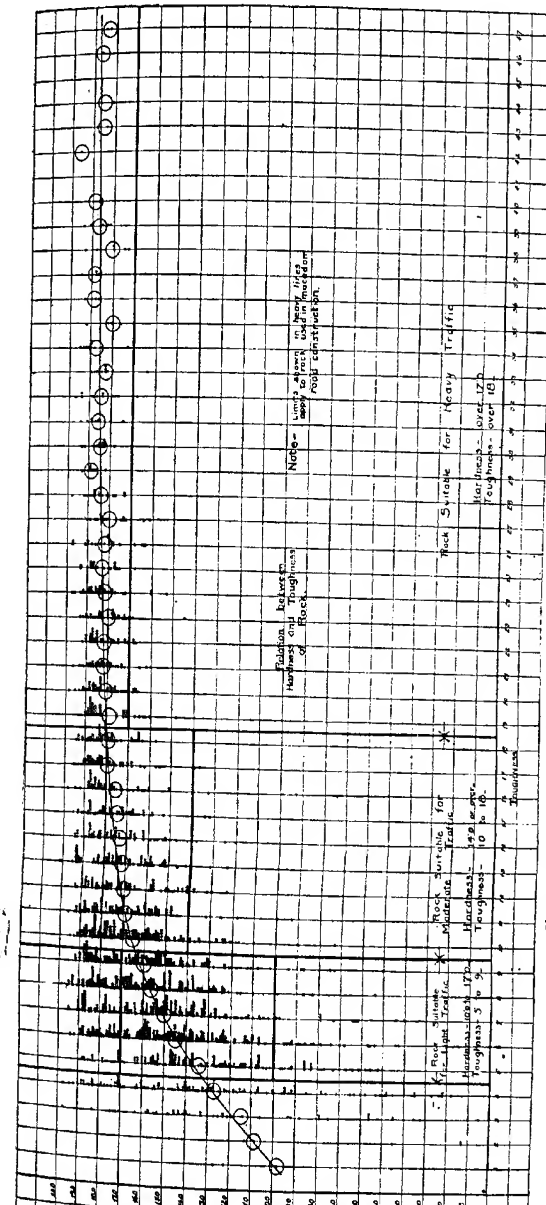

FIG. 1.—Curve showing the results of tests of about 3,000 samples of road-building rock.

corresponding limiting values for hardness at 10, 14, and 17. In other words, after making all allowances for variations due to local conditions, it may be fairly assumed that stone for use under light, horse-drawn, steel-tired vehicles should show a toughness of from 5 to 9 and a hardness of from 10 to 17; for moderate traffic a toughness of from 10 to 18 and a hardness of over 14, and for heavy traffic a toughness of 19 or over and a hardness of 17 or over. The terms "light," "moderate," and "heavy" in this connection refer to the total volume of traffic upon the road, calling, say, under 100 teams a day "light," 100 to 250 "moderate," and over 250 "heavy."

Practically all the values of hardness shown in figure 1 are above the various lower limits set by the best water-bound macadam-road practice. For light-traffic conditions, 94 per cent of all the samples tested have a hardness of more than 10; for moderate traffic, 95 per cent have a hardness of more than 14; and for heavy traffic, 94 per cent have a hardness of more than 17.

In other words, if it be assumed that the curve (fig. 1) represents a fair average of all available types of road-building rock, it would seem that a determination of the toughness of any particular sample of rock shows, for all practical purposes at least, whether it is hard enough to be satisfactorily used in construction.

If the curve be referred to again, it will be seen that a large number of hardness tests appear above the upper limit of 17 set for light-traffic conditions. Although on its face this would indicate that a determination of the hardness is necessary in this instance, reference to test records show that by far the greatest number of these tests (about 75 per cent) are on granites, quartzites, and hard sandstones, which are unsuited for use in the wearing course of water-bound macadam roads, owing to their lack of binding power, as shown by actual test.

Finally, the results of 2,500 individual routine tests made by the Office of Public Roads and Rural Engineering show that for practical routine work the hardness test adds nothing to our knowledge of the value of any particular rock sample for use in water-bound macadam-road construction over that obtained from the toughness test.

While the binding or cementing value of a rock is a most important consideration from the standpoint of ordinary macadam construction, the same is not true of broken-stone roads which are surface treated or constructed with an adhesive bituminous material. The hardness of the rock is also of relatively less importance, owing to the fact that the fine mineral particles produced by the abrasion of traffic combine or should combine with the bituminous material to form a mastic which is held in place and protects the underlying rock from abrasion so long as by proper maintenance it is kept intact. The toughness of the rock, however, is of more importance, as the shock of impact is to a considerable extent transmitted through the seal coat and may cause the underlying fragments

to shatter. It would therefore seem that the minimum toughness of a rock for use in the construction of a bituminous broken-stone road or a broken-stone road with a bituminous-mat surface should for light traffic be no less than for ordinary macadam subjected to the same class of traffic. For moderate and heavy traffic, however, the same minimum toughness may probably prove sufficient, owing to the cushioning effect of the bituminous matrix. No maximum limit of toughness need, however, be considered for any traffic.

In the case of bituminous concrete roads, where the broken stone and bituminous material are mixed prior to laying and consolidation, it would perhaps appear advisable to set a minimum toughness of 6 or 7 for light-traffic roads instead of 5, in order to insure against the possibility of the fragments of rock which have been coated with bitumen being fractured under the roller during consolidation, and of 12 or 13 for moderate and heavy traffic, instead 10 and 19, as in the case of water-bound macadam roads.

For broken-stone roads which are to be maintained with dust palliatives, it would appear that the same limits of toughness should hold as for ordinary macadam.

For easy reference the following limits of toughness are given in Table I, as representing facts developed in the foregoing discussion. It is, of course, quite probable that these limits will require modification as the correlation of laboratory tests to service results becomes more perfect.

Table I.—*Limits for toughness for rock used in the construction of broken-stone roads*

Type of road.	Light traffic.		Moderate traffic.		Heavy traffic.	
	Minimum.	Maximum.	Minimum.	Maximum.	Minimum.	Maximum.
Macadam Macadam with dust palliative	5	9	10	18	19	......
Macadam with bituminous mat Bituminous broken stone with seal coat	5	......	10	......	10	......
Bituminous concrete with or without seal coat	7	......	13	......	13	......